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EFFECT OF 1-AMINOCYCLOPROPANE-1-[1] CARBOXYLIC ACID, AND ETHREL ON GERBERA IN VITRO CULTURE

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ABSTRACT : Gerbera (*Gerbera jamesonii,* Bolus) plantlets in 125 ml flask received 0, 2.5, 5, 10, 20 ppm Ethrel and 0.01,0.1,1 mM l- aminocyclopropane-l- carboxylic acid (ACC) to study the effect of ethylene and ethylene biosynthesis through cultured in vitro. The good quality plantlets which had more adventitious roots, no overgown stem, no etiolated plantlets by the addition of 5, 10 ppm Ethrel. However, 20 ppm Ethrel caused etiolation of leaves and less roots. This result shows that 20 ppm Ethrel for gerbera plantlets was overdsage. The addition of ACC induced the deteriorated effect on the growth of plantlets. The damage of roots and stunted growth were observed in ACC treatment.

Key Words : *Gerbera jamesonii,* ethylene, 1-aminocyclopropane-1-carboxylic acid.

INTRODUCTION

Ethylene is produced by all higher plants and regulates a wide variety of phenomena plant growth and development (Abeles, 1973; Yang and Hoffman, 1984; Abeles, 1985). Adams and Yang elucidated the ethylene biosynthesis pathway in plants from S-adenosyl -methionine (SAM) via l-amino-cyclopropane-l- carboxylic acid(ACC) (Adams et al., 1979). LaRue and Gamborg, and Gavinlertvatana et al. reported that ethylene was produced in cell suspension culture but the amounts were low and evidently did not affect cell growth (LaRue and Gamborg, 1971; Gavinlertvatana et al., 1982). Moreover, Wochok and Wetherell found that Ethephon suppressed organized growth in cultured wild carrot tissue (Wochok and Wetherell, 1971), and Hsu amd Stewart reported that the Ethephon applied to an agar medium inhibited normal differentiation of cotton ovules and hastened callus formation (Hsu and Stewart, 1976). Recently, Babbar et al. found that the ethylene precursor methionine (MET) and the ethylene releasing compound, Ethrel, stimulated embryo formation and plantlet development. However, the ethylene antagonists cobalt and silver ions decrease the average number of embryos plants developed per anther in *datura* anther culture (Babbar and Gupta, 1986). Cho and Kasha found that the addition of ethylene promotors,

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Ethrel or ACC increased embryogenesis (Cho and Kasha, 1989). Huxter et al. reported ACC was supplied after 15 days in culture, and it had little or on effect on the rates of shoot primordium formation and long term treatments with higher levels of ACC inhibited shoot primordium formation (Huxter et al., 1981).

In this study, gerbera plantlets were cultured on agar medium with Ethrel and ACC content to study the effect of ethylene and ethylene biosynthesis through ACC in vitro. The initial objective was to investigate the possible role of ethylene during plantlet growth before their transplant from flasks. The coversion from ACC to ethylene will be examined from tissue after the plantlet absorbed ACC. The purposes of this study were to improve the quality of flask plantlets with healthy prosperous roots with more rooting, no overgrown stem, to find out the optimum concentration avoiding the etiolated plantlets which were caused by overdosage of Ethrel, and ACC.

MATERIALS AND METHODS

Plant materials

Gerbera (*Gerbera jamesonii*, Bolus, "Peach Spring") plantlets were placed on the culture medium. The plantlets were obtained from the meristem tissue culture. The size of plantlets was at average of 1-1.5 cm length including leaf and petiole.

The culture method

The plantlets were cultured in the medium containing Murashige and skoog salts (Murashige and Skoog, 1962) and the following, in mg/1: kinetin,1; NAA, 2; thiamine · HCl, 0.4; myo-inositol,100; sucrose 30,000; and Difco Bacto agar 7,000. Ethrel 2.5, 5, 10, 20 ppm and ACC 0.01, 0.1, 1 mM were added to the agar culture medium following millipore filter sterilization after all media were sterilized by autoclaving 15 min at 121°C. The pH of all media was set at 5.7. The media were dispensed into 125 ml pyrex flask at a rate of 25 ml. The flasks were closed with autocavable rubber septum. The plantlets were maintained at constant 25°C under 16-hr daily exposure to 9000 lux light. After 5 weeks the growth of roots and leaves of plantlets were recorded.

Ethylene measurement

For experiments in which ethylene production by the plantlets was determined. At the every end of 1 week incubation period, a 2 ml air sample in flask was analyzed by using gas chromatograph equipped with flame ionization detector.

ACC assay

The portions of leaves and petioles weighing 1 g were extracted twice with 5 ml of boiling 80% ethanol. The ethanol with the extracted residue was evaporated under vacuum at 55°C. The residues were dissolved in 2 ml of water. The ACC content in 0.3 ml aliquots of the aqueous solution was determined according to the method of Lizada and Yang (Lizada and Yang, 1979).

RESULTS AND DISCUSSION

Effect of Ethrel and ACC on the growth of plantlets

The number and diameter of roots were recorded (Table 1). The number of roots was increased at the most by Ethrel 5 ppm treatment and then declined (Fig. 1, 2). The results were same as a number of investigators have observed. The use of Ethrel to increase rooting of apple seedlings and blueberry seedling (Abeles, 1973). These observation represent an at-tempt to motivate propagation of important horticultural plants by ethylene-induced root fomation (Abeses, 1973). Nevertheless, ACC, the intermediate precursor of ethylene caused the damage of root growth (Fig. 3,4). The addition of ACC caused the decrease of rooting (Table 1). To convert ACC in plant tissue is very limited because the ethylene-forming enzyme can be induced just during certain developmental stages, especially at the climacteric stage (Yang, et al., 1985). There is little report about the conversion of ACC to ethylene in plantlet tissue in vitro. The accumulation of ACC may cause the phytotoxic effect due to the failure of conversion. It have to be investigated further. The leaf numbers were increased following the change as root change in Ethrel treatment (Table 2). This phenomenon have occured on lily, tulip (Abeles, 1973). Moreover, Ethrel caused curling of leaves as the previous report (Abeles, 1973). The addition of ACC caused less leaves. The mechanism is still unknown and has to be investigated.

Table 1. The effect of Ethrel and ACC on root growth of gerbera plantlets after 35 days in vitro. Values represent the mean ± SE of 5 replicates. The symbol of "—" means the death of plantlets.

Treatment		Root number	Root diameter
Control		7.4±1.5	0.03±0.01(cm)
Ethrel	2.5ppm	12.3±2.4	0.05±0.02
	5.0	18.1±2.2	0.07±0.03
	10.0	12.5±2.3	0.08±0.02
	20.0	6.4±1.6	0.04±0.01
ACC	0.01mM	5.2±2.2	0.04±0.02
	0.1	2.1±0.4	0.05±0.01
	1	—	—

Table 2. The effect of Ethrel and ACC on leaf growth of gerbera plantlets after 35 days in vitro. Values represent the mean ± SE of 5 replicates. The symbol of "—" means the death of plantlets.

Treatment		leaves	length
Control		6.3±1.6	8.95±0.18(cm)
Ethrel	2.5ppm	7.6±2.1	8.37±0.22
	5.0	10.2±2.3	6.79±0.16
	10.0	7.2±1.4	4.45±0.84
	2.0	6.1±1.4	3.26±0.72
ACC	0.01mM	7.4±1.2	3.15±0.72
	0.1	6.1±1.4	2.94±0.42
	1	—	—

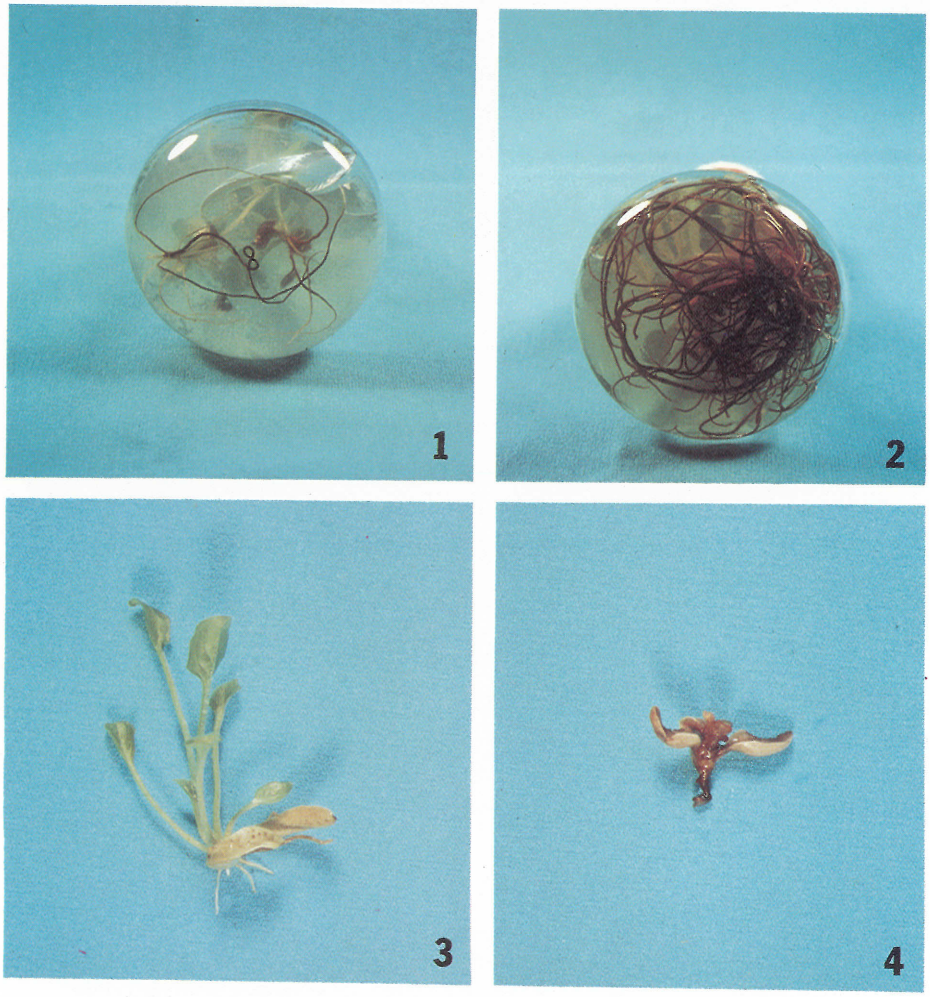

Fig. 1. The gerbera plantlets grown in vitro without Ethrel are showed long root and less rooting.
Fig. 2. The well grown rootings are showed in the medium with Ethrel 5 ppm after 5 weeks cultured in vitro.
Fig. 3. The deleterious effect of ACC on gerbera plantlet after cultured with ACC 0.01 mM in medium for 5 weeks.
Fig. 4. ACC 1 mM caused the death of gerbera plantlet cultured in vitro.

Ethylene production from the addition of Ethrel

The cumulative release of ethylene was proportional to the Ethrel concentration of the nutrient medium (Fig. 5). The 5 eeks amount of ethylene were 35 nl/l for Ethrel 2.5 ppm; 114 nl/l for 5 ppm; 205 nl/l for 10 ppm; 295 nl/l for 20 ppm ; and 30 nl/l for control. The ethylene production was proportional to Ethrel concentration (Fig. 6). Ethrel could be attributed to a volatile component, ethylene, amd the significant suppression of plantlet growth and the increase of root number were associated with the ethylene production which was liberated from Ethrel (Table 1, 2, and Fig. 5). Tisserat et al. showed that the ethylene which liberated by the decomposition of Ethephon suppressed asexual

embryogenesis of *Daucus carota* L. (Tisserat and Murashige, 1977). It was also reported that the ethylene liberated in the cultures in direct proportion to Ethephon added to the medium (Tisserat and Murashige, 1977). In this study, Ethrel also played the role of ethylene on gerbera plantlet in vitro.

Ethylene production from the addition of ACC

A progressive increase of ethylene was associated with increasing concentration of ACC (Table 3). However, the increasing consecutive residue of ACC was associatd increasing concentration of ACC treatment (Table 4). The conversion of ACC to ethylene was obviously incomplete. Yang et al. reported that a massive increase in ethylene production requires development of both ACC synthase and ethylene-forming enzyme (Yang and Hoffman, 1984). However, ethylene-forming enzyme can be induced during certain developmental stage, around the climacteric peak of ethylene production (Yang et al., 1985). The higher ACC residue in plantlet tissue showed the more serious phytotoxic effect on the retardation of growth and less rooting (Fig. 3, 4). Huxter et al. showed that the long term (3 weeks) treatments with higher level (500 uM) of ACC inhibited shoot primordium formation on tabacco (Huxter et al., 1981). In this study, the supply of ACC only at the rate 1 mM caused the death of plantlets during the first 2 weeks, and 0.01 mM showed the retardation of plantlets associated the ethylene production which was liberated from ACC at the rate of 54. 4 nl/l after 5 weeks in vitro culture (Table 3). In this study, the gerbera plantlets might be sensitive to ACC, and lack of enough ethylene-forming enzyme to convert ACC ethylene. It has to be investigated further.

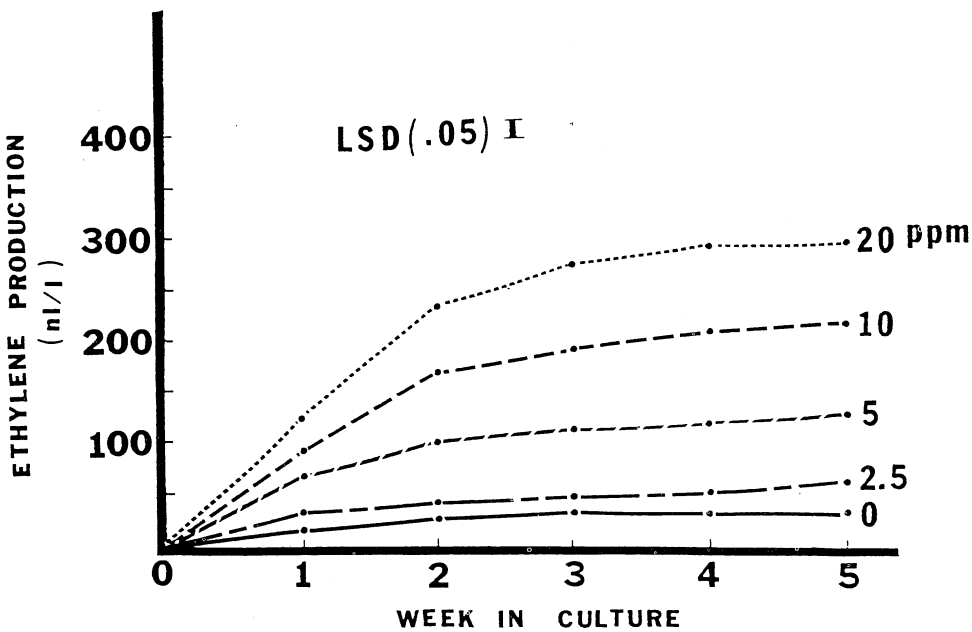

Fig 5. Ethylene production of gerbera plantlets treated with various concentration of Ethrel in vitro.

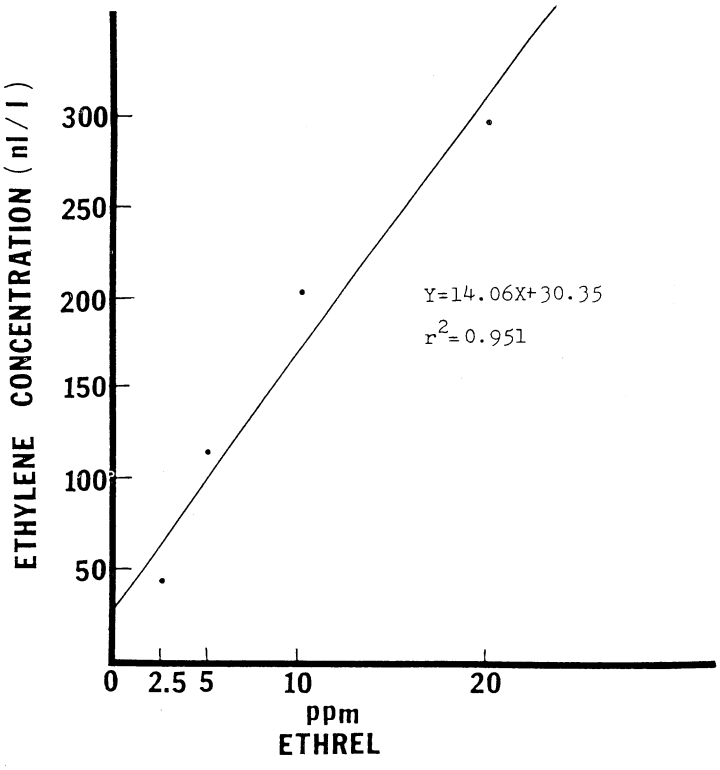

Fig 6. Ethylene cumulative concentration of gerbera plantlets treated with various concentration of Ethrel in vitro after 5 weeks.

Table 3. Ethylene production by gerbera plantlets treated with ACC in vitro. Values represent the mean ± SE of 5 replicates. The symbol of "—" means the death of plantlets.

Week / Treatment	Ethylene production (nl/l)				
	1	2	3	4	5
Control (no ACC)	7.2±1.2	24.3±0.8	31.7±0.6	32.4±0.3	34.7±0.5
ACC 0.01mM	11.3±0.3	38.5±0.2	39.2±0.4	47.6±0.4	54.4±0.7
0.1	13.3±0.9	42.6±0.4	52.6±0.9	69.4±0.8	74.5±0.7
1	19.8±0.7	—	—	—	—

Table 4. Residue of ACC in gerbera plantlet tissue treated with the addition of ACC in vitro. Values represent the mean ± SE of 5 replicates. The symbol of "—" means the death of plantlets.

Week / Treatment	ACC residue(nmol/g)				
	1	2	3	4	5
Control (no ACC)	2.1±0.4	3.8±0.3	4.0±0.5	4.2±0.4	4.5±0.3
ACC 0.01mM	2.6±0.4	4.4±0.5	4.8±0.2	5.2±0.3	5.9±0.4
0.1	2.8±0.3	4.7±0.4	5.0±0.7	5.4±0.6	6.3±0.5
1	5.7±0.4	—	—	—	—

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環丙烷基羧酸與益收素對試管內非洲菊之影響[1]

林 瑞 松[2]

摘 要

非洲菊 (*Gerbera jamesonii,* Bolus) 幼苗於試管內處理以0, 2.5, 10, 20 ppm 的益收素 (Ethrel) 及 0.01, 0.1,1 mM 的環丙烷氨基羧酸 (l-aminocyclopropane-l-carboxylic acid, ACC) 以研究乙烯對試管苗之影響及乙烯生合成，結果以5及10ppm的益收素處理能得到良好品質的苗，爲多側根不徒長，不白化的幼苗。但是 20 ppm 的益收素造成葉片白化根數少之過量危害。ACC 的作用 (註 ACC 是乙烯之中間先驅物質)，造成對幼苗不利於生長，根部危害甚大停止生長。此現象顯現於全部 ACC 的處理。

關鍵語：非洲菊、益收素、環丙烷基羧酸。

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